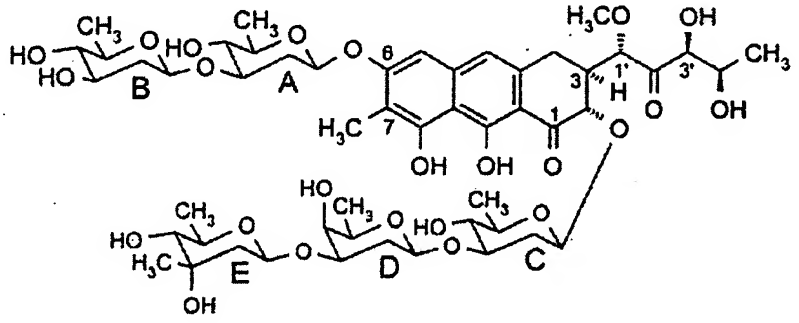
AMENDMENT TO THE SPECIFICATION

Please amend paragraphs [0002], [0003], [0012], [0028], [0037], [0040], [0045], [0051], [0065], [0066] and [0077] of the specification and the Abstract to read as follows:

[0002] This application claims the benefit of priority of Provisional Application No. 60/548,175 ~~(unknown)~~, filed March 1, 2004 which is incorporated herein by reference.

[0003] Mithramycin (MTM) is an aureolic acid-type polyketide produced by various soil bacteria of the genus *Streptomyces*, including *Streptomyces argillaceus* ATCC 12956 (deposited with ATCC, P.O. Box 1549, Manassas, VA 20108 USA). MTM has the following formula (I):



(I)

MTM is the most important representative of the aureolic acid group of antitumor agents, and is used to treat testicular carcinoma, Paget's disease and hypercalcemia caused by malignancy-associated bone lesions. MTM is also an agent for neuroprotection in the treatment of neurological diseases such as stroke, amyotropic lateral sclerosis, Parkinson's disease, Huntington's disease, multiple sclerosis and viral encephalitis.

[0012] Fig. 1 is the gene organization of the MTM biosynthetic gene cluser in *Streptomyces[[.]] argillaceus*.

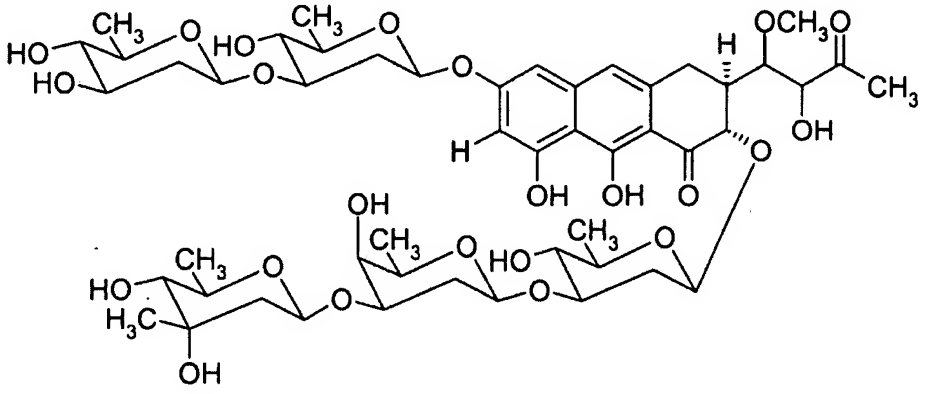
[0028] The *mtmW* [[a]] gene is located ca. 8 kb downstream of the mithramycin PKS genes. The gene is replaced by an *aac(3)IV* gene that yields a *S. argillaceus* mutant, which produces four new mithramycin derivatives, namely mithramycin-SK, demycarosyl-mithramycin-SK, mithramycin-SA, mithramycin-SDK. Mithramycin-SK is the major product. The structures of mithramycin-SK and demycarosyl-mithramycin-SK bear a butyl side chain attached at carbon 3 instead of the expected pentyl side chain with an additional keto function. This can be explained through a non-enzymatic Favorskii-type rearrangement of the initially formed pentyl side chain with two keto groups in β-position to each other. Fig. 3 shows the non-enzymatic Favorskii-type rearrangement in the formation of mithramycin-SK and mithramycin-SA having structures **2** and **4**, respectively, from MTM having structure **5**.

[0037] The method for making and isolating derivatives mithramycin-SK, mithramycin-SA, demycarosyl-mithramycin-SK, and mithramycin-SDK, generally involves (i) incubating the mutant *S. argillaceus* M7W1, (ii) forming a composition and (iii) isolating the derivatives from the composition. The incubation time and temperature will vary depending upon the amount of mutant that is employed. The incubation temperature is generally from 25 °C to 40 °C, from 30 °C to 35 °C, and preferably 30 °C. The incubation time ranges from generally several hours to several days, e.g., from 1 to 10 days, 2 to 9 days, 3 to 8 days, 4 to 7 days, and 5 to 6 days.

[0040] When plasmid-containing clones were grown, the medium was supplemented with the appropriate antibiotics: thiostrepton, 25 μg/mL; tobramycin, ~~20 /μg/mL~~ 20 μg/mL; ampicillin, 100 μg/mL; or apramycin, 25 μg/mL. Plasmids pBSKT, pIJ2921, pIAGO, and pEFBA were used (the pEFBA plasmid is a pBSK derivative containing an apramycin resistance cassette; pBSK can be obtained from Stratagene, 11011 M. Torrey Pines Road, La Jolla, CA). Plasmid DNA preparations, restriction endonuclease digestions, alkaline phosphatase treatments, ligations, Southern hybridization, and other DNA manipulations were performed according to standard procedures for *E. coli* and *Streptomyces*.

[0045] The *aac(3)IV* gene is deposited at Gen Bank having accession numbers [[are]] X01385 and V01499. The sequence listing containing the *aac(3)IV and mtmW* genes is set forth in Fig. [[56]] 6 as SEQ ID NO:1. Nucleotides 1416 to 4221 of SEQ ID NO:1 represent the mutated *mtmW* gene while nucleotides 2104-3608 of SEQ ID NO:1 represent the *aac(3)IV* cassette. This construct, pM7W1, was used to transform protoplasts of *Streptomyces argillaceus*, and these transformants were selected for resistance to apramycin. Any antibiotic resistance gene can be used provided that it can be selected for resistance in *Streptomyces argillaceus*. Examples include, but are not limited to, erythromycin, hygromycin, thiostrepton, spectinomycin, viomycin and kanamycin.

[0051] An ~~anoalog~~ analog of mithramycin-SK having the following formula (VII) can be produced from mutant *S. argillaceus* M7W1:



(VII)

[0065] Compilation of the average log(GI_{50}) values showed that both compounds were active, with mithramycin-SK (activity up to 9 times higher than that of MTM) being much more active than demycarosyl-mithramycin-SK (ca.25 times less active than MTM). Mithramycin-SK was particularly active against melanoma, leukemia, and CNS cancer cells (log(GI50) values of -7.64, -7.59, and -7.61, respectively). Given the increased activity observed for mithramycin-SK, a neutral red uptake analysis of squamous, melanoma, lung, and breast carcinomas was

performed, which not only confirmed the increased activity of mithramycin-SK as compared to MTM, but also showed an even more pronounced improvement of activity (up to ca. 90 times better). In addition, toxicity assays using this same process and mouse 3T3 fibroblast (nontumor) cells showed that 2, with an IC_{50} value of 1.96×10^{-5} M, is more than 1500-fold less toxic than MTM (IC_{50} values ranging from 1.29×10^{-8} to 3.45×10^{-9} M). Thus, mithramycin-SK displays a significantly improved therapeutic index, up to 4 orders of magnitude better when compared to its parent compound, MTM. The results are shown in Table 4.

Table 4. Antitumor Analysis Comparing Mithramycin **(1)**, Mithramycin SK **(2)**, and Demycarosyl-Mithramycin-SK **(3)**.

Type of Cancer	**1**	**Composition with 2**			**Composition with 3**		
		2	**Δ_{1-2}**	**AIF[a]**	**3**	**Δ_{1-3}**	**AIF**
		Average Log(GI_{50}) Values from Sulforhodamine B Assay					
Leukemia (5)[b]	-6.65	-7.59	0.94	8.7	-5.55	-1.10	0.08
NSCLC (8)[b]	-6.73	-7.37	0.64	4.4	-5.30	-1.43	0.04
Colon (7)[b]	-6.65	-7.32	0.67	4.7	-5.35	-1.30	0.05
CNS (5)[b]	-6.78	-7.61	0.83	6.8	-5.30	-1.48	0.03
Melanoma (8)[b]	-6.72	-7.64	0.92	8.3	-5.37	-1.35	0.04
Ovarian (6)[b]	-6.60	-7.53	0.93	8.5	-5.23	-1.37	0.04
Renal (8)[b]	-6.73	-7..29	0.56	3.6	-5.14	-1.59	0.03
Prostrate (2)[b]	-6.90	-7.48	0.58	3.8	-5.25	-1.65	0.02
Breast (8)[b]	-6.59	-5.89	-0.70	0.2	-5.15	-1.44	0.04
	Average Log(GI_{50}) Values from Neutral Red Assay						
Squamous carcinoma	-5.04	-5.99	0.95	8.9			
Melanoma	-5.05	-6.25	1.20	15.8			
Lung carcinoma	-4.92	-6.88	1.96	91.2			
Breast carcinoma	-4.95	-6.74	1.79	61.6			

[a] Activity improvement factor. This factor is equal to $10^{\Delta 1-x}$, where x is the identifying value for compound **2** or **3**. An AIF of 1.0 corresponds to no difference in activity.

[b] The number in parenthesis is the number of cell lines tested in each family.

The data in Table 4 shows that mithramycin-SK (compound **2**) exhibits an ~~anticity~~ activity that is up to 90 times higher than MTM (compound **1**).

[0066] The dosages or amounts of the compounds of the invention are large enough to produce the desired effect in the method by which delivery occurs. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the subject and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician based on the clinical condition of the subject involved. The dose, schedule of doses and route of administration may be varied. Doses and dosing regimens used form Mithramycin provide guidance for [[does]] dose and dosing regimens for Mithramycin SK (see for example Trask and Sonhami, "Effect of Mithramycin on Widespread Painful Bone Metastases in Cancer of the Breast," *Cancer Treat. Rep.*, 63(11-12): 1835-1838 (1979); and Conrad et al., "Mithramycin in the Treatment of Systemic Mastocytosis," *Ann. Intern. Med.*, 83(5): 659-660 (1975)). For example, a single or multiple dose can be administered. In one embodiment, the dosages can be in ranges from 0.1 to 100 mg/kg, 0.1 to 90 mg/kg, 0.1 to 80 mg/kg, 0.1 to 70 mg/kg, 0.1 to 50 mg/kg, 0.1 to 20 mg/kg, 0.1 to 10 mg/kg, 0.1 to 5 mg/kg or 0.1 to 1 mg/kg. In another embodiment, the compounds of the invention can also be administered for 5 days with a daily [[does]] dose of 0.12 mg/kg. In yet another embodiment, a single [[does]] dose of 1.0 mg/kg to 10 mg/kg can be administered.

[0077] The present invention also provides a method of providing neuroprotection in subject ~~diagnose~~ diagnosed with neurological diseases, the method comprising the step of administering to the subject an effective amount of a compound of the invention in a pharmaceutically acceptable carrier, whereby the compound provides neuroprotection in the subject. The subject can be a mammal, preferably a human, and the compound is administered parenterally.